Osteoarthritis and Cartilage (2000) **8,** 186–196
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doi:10.1053/joca.1999.0289, available online at http://www.idealibrary.com on IDEAL®
1063–4584/00/030186+11 $35.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

Diacerhein and rhein reduce the ICE-induced IL-1β and IL-18 activation in human osteoarthritic cartilage

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Summary

Objective: IL-1β plays a fundamental role in osteoarthritis (OA) pathophysiology and cartilage destruction. Targeting the activation mechanism of this cytokine appears to be important as a therapeutic approach. As the interleukin-1 converting enzyme (ICE) is the physiologic modulator of the production of active IL-1β, we investigated the effect of diacerhein and its active metabolite rhein used in the treatment of OA patients, on the enzyme expression and synthesis on human OA cartilage. Further, we looked at the effect of both drugs on the production of the active form of IL-1β and IL-18.

Methods: The expression and synthesis of ICE were investigated on human OA cartilage explants using in-situ hybridization and immunohistochemical methods, respectively. The effect of the drugs on ICE OA chondrocytes was also determined by Northern blotting and a specific ELISA assay. Furthermore, the effect of both drugs on the level of active IL-1β and IL-18 was examined by immunohistochemistry.

Results: Data showed that diacerhein and rhein have no true effect on reducing total ICE mRNA by both Northern blotting analysis and in-situ hybridization. A marked and statistically significant decrease was, however, found for protein production. ELISA showed a reduction of 31% ($P<0.04$) for diacerhein and 50% ($P<0.02$) for rhein. The drugs' immunohistological cell score reduction was similar to data from the ELISA, and a statistical significant reduction of ICE production was found at both superficial and deep zones of the cartilage. IL-1β and IL-18 were both preferentially produced in chondrocytes of the superficial zone. For each of these cytokines, both drugs demonstrated a statistically significant decrease in this zone. A marked decrease was also noted in the deep zone, but statistical significance was reached only for rhein.

Conclusion: These results provide a novel regulatory mechanism by which diacerhein and rhein could exert a down-regulation on IL-1's effect on OA cartilage. © 2000 OsteoArthritis Research Society International

Key words: OA cartilage, Diacerhein, ICE, Interleukin-1β, Interleukin-18.

Introduction

Osteoarthritis (OA) is a multifactorial disease characterized by a progressive degradation of articular cartilage. Articular cartilage is an avascular tissue in which the chondrocytes are embedded in an abundant extracellular matrix, composed mainly of type II collagen, aggrecans, glycoproteins and other minor components. The integrity of the tissue structure is maintained by a balanced control between anabolic and catabolic processes, which is regulated by ambient growth factors and cytokines.

Numerous studies from either in-vitro or in-vivo experiments have established the key role of IL-1β in the OA process.[1–7] Several cells including macrophages, synovial cells and chondrocytes in articular joint tissues produce IL-1β. This cytokine contributes to the degeneration of articular cartilage by stimulating the cells to produce proteolytic enzymes (in cartilage, the most important are the metalloproteases—collagenases, stromelysins and gelatinases), but also by decreasing the anabolism of the chondrocytes (see review[8]).

IL-1β is synthesized as a biologically inactive precursor and requires a proteolytic cleavage to be active.[9–11] This is achieved by a highly selective protease, the IL-1 converting enzyme (ICE), also called caspase-1.[12,13] This enzyme, ICE, has another cytokine as a specific substrate, the IL-18 or interferon gamma inducer.[14,15] IL-18 has structural similarities to the IL-1 protein family and is produced as a pro-cytokine.[16,17] ICE is an endopeptidase localized intracellularly. This enzyme is synthesized as a 45 kDa proenzyme and the conversion into an active form involves autoproteolysis.[13,18] The active enzyme is formed as an heterodimer composed of two subunits of 20 and 10 kDa, both of which are required for catalytic activity.[19–21]

Our laboratory has recently shown that ICE is expressed and synthesized by both human synovial membrane and cartilage, with a marked increase in OA tissues over normal.[22] In OA cartilage, ICE appears to play a crucial role in IL-1β and IL-18 maturation, as ex-vivo treatment of cartilage explants with a specific ICE inhibitor (YVAD) completely abrogated the production of active IL-1β and markedly reduced the production of IL-18.[22] These findings, combined with others showing that transgenic ICE-deficient mice had major defects in the production of mature IL-1β and IL-18.[23,24] give credence to the use of

Received 17 August 1999; accepted 19 November 1999
This study was supported by a grant from Negma-Steba International Development N.V., The Hague, Netherlands.
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ICE inhibitor as a therapeutic target of IL-1 mediated diseases, including OA.

Diacerhein is a drug belonging to the anthraquinone chemical class and employed in OA treatment. This drug is a low molecular weight heterocyclic compound designated as 4,5-bis (acetyloxy)-9,10-dioxo-2 anthracene) carboxylic acid. Its mechanism of action appears to be different from that described for a classical nonsteroidal anti-inflammatory drug, in which diacerhein and its active metabolite rhein inhibit IL-1 production and activity and, depending on the cell type, either did not affect prostaglandin synthesis (macrophages) or stimulated its synthesis (chondrocytes).[25–31] In animal models of OA, diacerhein has demonstrated protective effects on cartilage matrix degradation.[32–35] In clinical trials, its oral administration was associated with symptomatic improvement in the majority of patients with OA.[36–38]

As IL-1β plays a fundamental role in OA pathophysiology and cartilage destruction, targeting the activation mechanism of this cytokine appears to be of importance as a therapeutic approach. As ICE is the physiologic modulator of the production of active IL-1β, we first investigated the effect of diacerhein and rhein on this enzyme's expression and synthesis on human OA cartilage. Secondly, we looked at the effect of both drugs on the production of the active forms of IL-1β and IL-18.

Material and methods

SPECIMEN SELECTION

Cartilage specimens (tibial plateaus) were obtained from OA patients (12F/7M; 72±7 mean age±SD) undergoing total knee joint replacement. The diagnosis was established according to the American College of Rheumatology criteria.[39] The specimens were obtained under aseptic conditions and processed as previously described.[22,40] Briefly, the cartilage was dissected from the underlying bone. Areas of fibrocartilage were identified and excluded.

In a first set of experiments, cartilage explants (about 100 mg) were dissected, rinsed in cold saline solution, and incubated for 24 h at 37°C in a humidified atmosphere of 5% CO_2/95% air in medium containing Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL Canadian Life Technologies, Burlington, Ontario, Canada), 2.5% heat-inactivated fetal calf serum (FCS; Hyclone, Logan, UT), antibiotics (penicillin, 100 U/ml; streptomycin 100 μg/ml; Gibco-BRL), in the presence or absence of 20 μg/ml of diacerhein or rhein (Negma Laboratories, Tossus Le Noble, France). The dosage use is within the range of physiological blood levels measured in patients treated with diacerhein.[41] Following the incubation, cartilage specimens were processed for either in-situ hybridization or immunohistochemistry.

In another set of experiments, chondrocytes were released from the cartilage by sequential enzymatic digestion as described:[22,30] 1 h with 2 mg/ml pronase followed by 6 h with 1 mg/ml collagenase (type IV, Sigma, St. Louis, MO) at 37°C in DMEM with 10% FCS and antibiotics. The digested tissue was centrifuged and the pellet washed. The isolated chondrocytes were seeded at high density in tissue culture flasks and cultured in DMEM supplemented with 10% FCS and antibiotics at 37°C in a humidified atmosphere. At confluence, the cells were detached and passaged once, then seeded at high density in the required flasks, and were allowed to grow for 5 days in DMEM supplemented as above. The culture medium was changed every second day and, 24 h before the experiment, the cells were incubated in fresh medium containing 2.5% FCS. Chondrocytes were then incubated with 20 μg/ml of diacerhein and rhein. Following the 24-h incubation period, cells were processed for mRNA or protein determinations (see below).

ICE EXPRESSION

RNA extraction and Northern blotting

Total RNA was extracted from chondrocytes using the Trizol reagent (Gibco-BRL) as previously described,[22,42] and processed according to the manufacturer's specifications. The extracted RNA was quantitated spectrophotometrically. For Northern blot, 20 μg of total RNA was resolved on 1.2% formaldehyde-agarose gels and transferred electrophoretically to nylon membranes (Hybond-N; Amersham Pharmacia Biotech, Oakville, Ontario, Canada) in a 10 mM sodium acetate buffer (pH 7.8) containing 20 mM Tris and 0.5 mM EDTA overnight at 4°C. The RNA was cross-linked to the membranes by exposure to ultraviolet light.

The human ICE probe was as previously described.[22] The primers were: 5′-GTTCCATGGGTGAAGGTACA-3′ (sense) corresponding to position 44–63 bp of the published sequence of the human gene;[43] and 5′-AATGAGAGCAAGACGTGTGC-3′ (antisense) from position 482–501 bp. Using these primers, a 456 bp fragment was obtained and cloned directly into the pCRII vector (Invitrogen, San Diego, CA).

In-situ hybridization

Cartilage specimens were processed as previously described[22] using ICE RNA probe labeled with digoxigenin-UTP (DIG-UTP, Boehringer Mannheim, Indianapolis, IN). Briefly, cartilage samples were fixed in 4% paraformaldehyde and embedded in paraffin. Five micron sections were deparaffinized, hydrated, incubated in 0.3% Triton X100/PBS and digested using 0.1% pepsin (Boehringer Mannheim) in 0.2 M HCl, and 10 μg/ml proteinase-K (Sigma) in 100 mM Tris-HCl and 50 mM EDTA pH 8. Each slide was rinsed with 0.1% glycine/PBS (pH 7.4. 22°C), and post-fixed in 4% paraformaldehyde for 5 min. Slides were acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine pH 8, then immersed in prehybridization buffer: 50% deionized formamide with Dower® MR, 1× Denhardt's solution, 0.5% denatured salmon sperm DNA, 0.25% yeast tRNA and 5% dextran sulfate (all from Sigma). The slides were then rinsed with 2×SSC and hybridized with the DIG-labeled RNA-antisense probe (or DIG-labeled RNA-sense probe for controls) at 1 μg/ml in the prehybridization buffer. Following overnight incubation with the probe at 46°C, the slides were washed and digested with 20 μg/ml RNase-A (Sigma) for 30 min at 37°C. After washing in SSC, the cartilage specimens were processed using the anti-DIG POD Fab fragments (1.5 U/ml; dilution 1/100; Boehringer Mannheim) and the color was developed by 3′-3′ diaminobenzidine (DAB; Dako Diagnostics Canada Inc., Mississauga, Ontario, Canada) and hydrogen peroxide. Slides were counterstained with nuclear fast red stain (Digene Diagnostics, Silverspring, MD). All specimens were examined on a Leitz periplan photomicroscope, and micrographs taken using Kodak Ektachrome 64 color film.

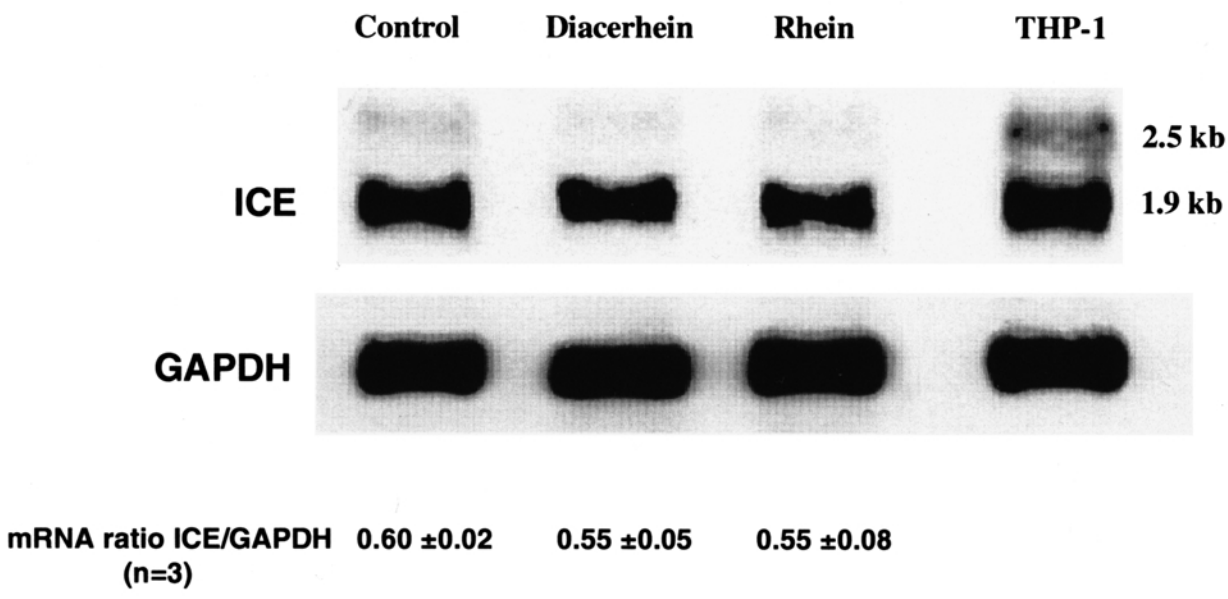

Fig. 1. Representative Northern blot of OA chondrocytes incubated in the absence (control) or presence of diacerhein and rhein at 20 μg/ml. The ratio indicates the mean±SEM of three independent experiments, and refers to the relative expression of ICE mRNA after being normalized to GAPDH. mRNA extracted from the human monocyte cell line THP-1 is also represented.

The evaluation of chondrocytes staining positive was performed using our previously published methods.[22,40,44] The expression of ICE was estimated by determining the number of cells staining positive within the different layers of tissue. For each specimen, six microscopic fields were examined (×40; Leitz Diaplan): three fields at the superficial and upper intermediate layers (superficial zone), and three fields at the lower intermediate and deep layers (deep zone). The total number of chondrocytes and the number of chondrocytes staining positive for ICE using the antisense probe were evaluated separately for each zone of cartilage and for the full-thickness cartilage (superficial and deep zones). Each slide was subjected to a double-blind evaluation, with a maximum variation of 5% recorded. Results were determined by the percentage of chondrocytes staining positive for ICE (cell score), the maximum score being 100%.

The ICE RNA probe was constructed as detailed in the RNA extraction and Northern blotting section, transcribed, labeled with the DIG-11-UTP, and revealed using a luminescent reagent.[22]

ICE PROTEIN DETERMINATION

ICE ELISA

For the quantitative determination of human ICE in chondrocytes, an ELISA kit from Cistron Biotechnology (Pine Brook, NJ) was used. After being isolated, chondrocytes were incubated for 24 h in the absence or presence of diacerhein and rhein at 10 and 20 μg/ml. After washing with ice-cold PBS, the chondrocytes were scraped and resuspended in a lysis buffer (10 mM Tris-HCl, 5 mM $CaCl_2$, 0.25% Triton X-100, 1 mM PMSF, 10 μg/ml pepstatin, 10 μg/ml leupeptin, pH 7.5). This ELISA is a monoclonal polyclonal based assay that is specific for the p20 subunit and recognizes both the precursor and active forms of ICE. The antiserum used in this assay showed specificity to human ICE, and no cross-reactivity was found with the cytokines, such as IL-1β and caspase-3, -7 or -8.

Immunohistochemistry

Cartilage specimens were processed for immunohistochemistry as previously described.[22,40,44] Specimens were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5 μ) of paraffin-embedded specimens were deparaffinized in toluene, dehydrated in a graded series of ethanol, and preincubated at 65°C for 20 min in 10 mM sodium citrate buffer pH 6.0 (when using ICE antibody) or with chondroitinase ABC (0.25 U/ml in PBS; Sigma) for 60 min at 37°C (when using IL-1β and IL-18 antibodies). Slices were then washed in PBS, followed by 0.3% hydrogen peroxide/methanol. They were further incubated with 2% normal serum (Vector Laboratories, Burlingame, CA) and overlaid with the antibodies for 18 h at 4°C in a humidified chamber. The evaluation of chondrocytes staining positive was performed as detailed above for in-situ hybridization.

The antibody used for ICE was an affinity-purified rabbit polyclonal antibody raised against a peptide corresponding to epitope 2-20 mapping at the caraboxy terminus of the ICE precursor of human origin and termed P20 subunit (#sc-1780; 10 μg/ml; Santa Cruz Biotechnology Inc., Santa Cruz, CA). This antibody recognizes both the precursor and active forms of ICE. The IL-1β antibody used was a monoclonal anti-human IL-1β (#MAB201, 10 μg/ml; R&D Systems Inc., Minneapolis, MN) that recognizes the mature (active) form of IL-1β and shows only minimal cross-reactivity with pro-IL-1β in biological fluid. The IL-18 antibody used was a goat polyclonal raised against a peptide corresponding to the amino acids 175-193 mapping at the carboxy terminus of the IL-18 precursor (#sc-6177, 5 μg/ml; Santa Cruz). This antibody recognizes both the precursor and active forms of IL-18, and has no cross-reactivity with

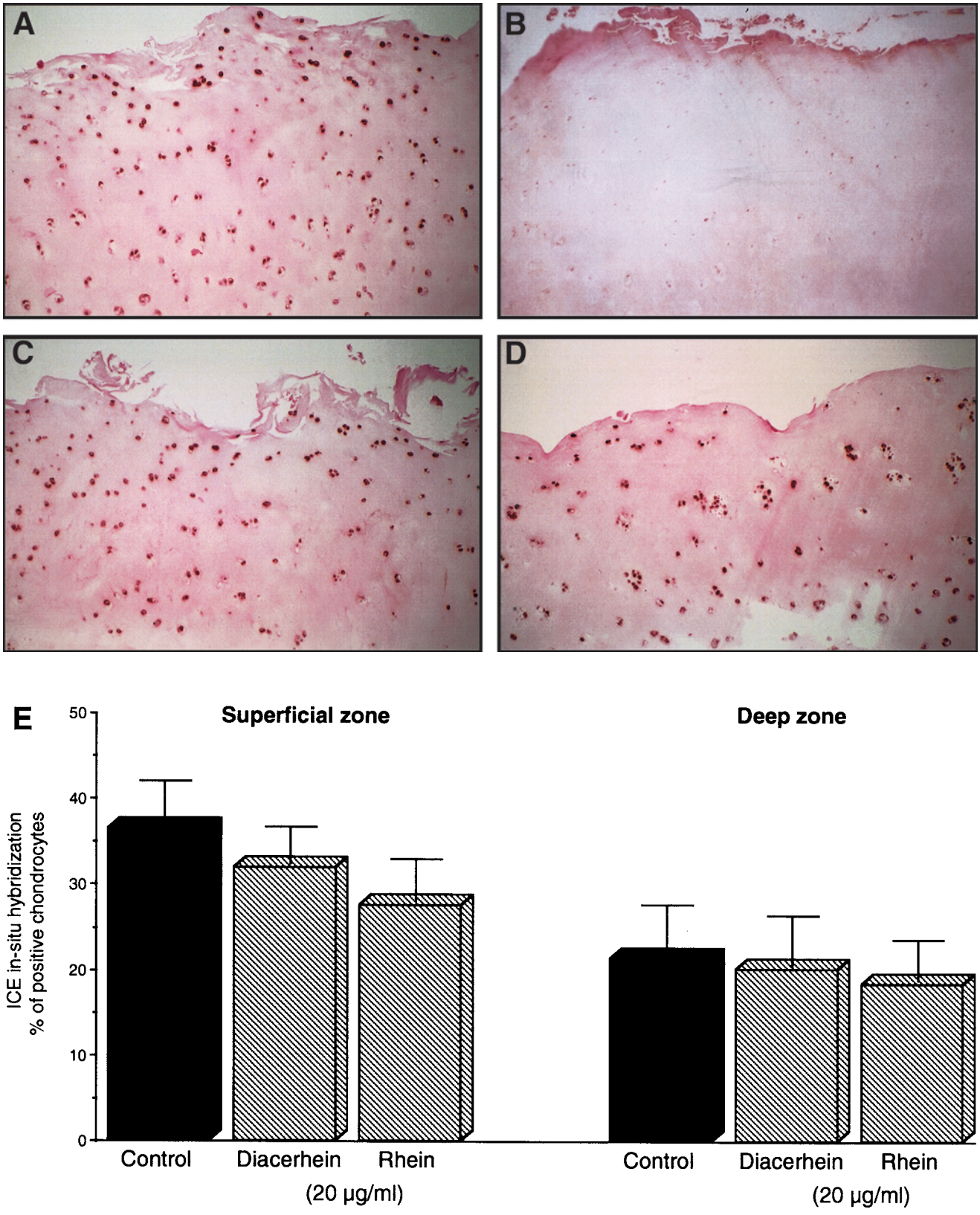

Fig. 2. Representative sections of ICE in-situ hybridization in human OA cartilage labeled with antisense (A) or sense as control (B) mRNA probe. (C, D) represent OA cartilage treated with diacerhein (C) or rhein (D) at 20 µg/ml. In-situ hybridization was carried out using an ICE mRNA antisense probe labeled with dDIG-11-UTP. (Original magnification ×63.) (E) Histogram of the cell score for ICE mRNA in OA cartilage from the superficial (superficial and upper intermediate layers) and deep (lower intermediate and deeper layers) zones. Data are expressed as the mean±SEM cell score. Statistical analysis revealed a significance difference (P<0.001) between the OA controls from the superficial and deep zones.

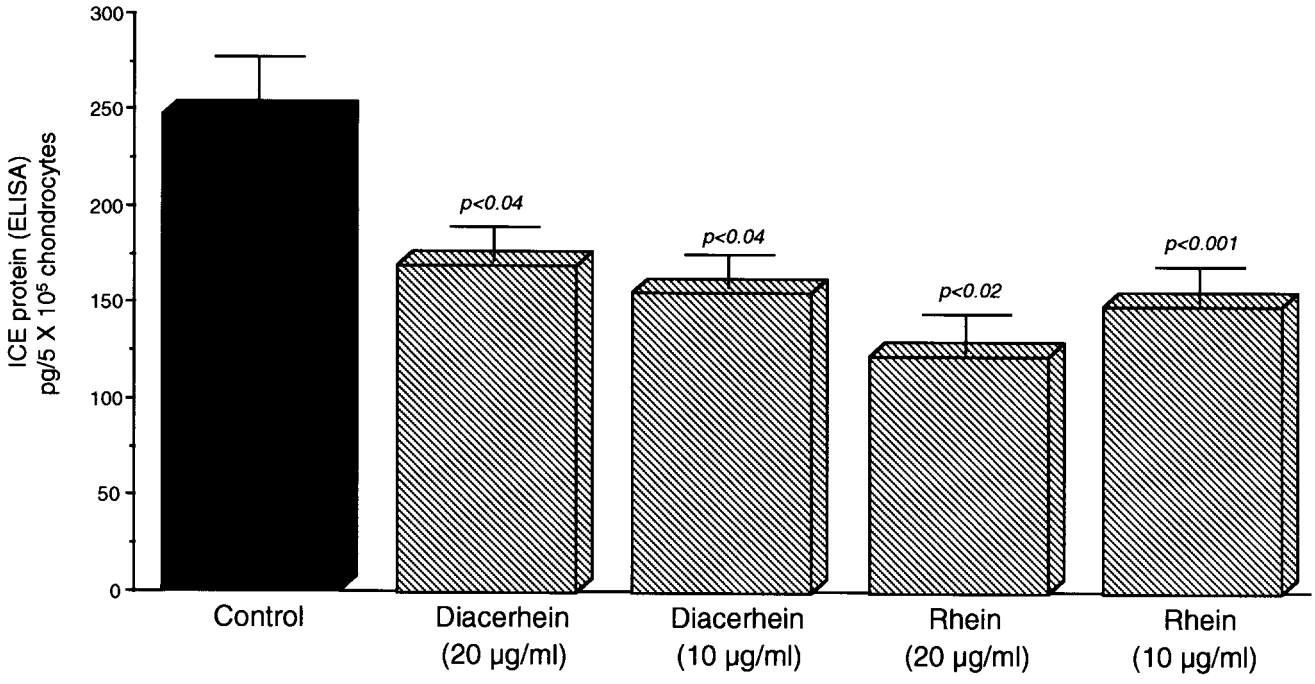

Fig. 3. ICE protein determination in human OA chondrocytes using a specific ELISA assay. Cells were incubated in the absence (control) or presence of diacerhein or rhein at 20 µg/ml and 10 µg/ml. Data are expressed as the mean±SEM cell score. *P*-values indicate comparison with the OA control.

other interleukins (Santa Cruz), and more specifically with IL-1β (personal observation).

Each slide was washed three times in PBS, pH 7.4, and stained using the avidin-biotin complex method (Vectastain ABC kit; Vector Laboratories). The color was developed by the DAB (Dako Diagnostics) containing hydroxide peroxide and the slides counterstained with nuclear fast red stain (Digene Diagnostics Inc.). The counterstaining gives light pink-stained nuclei, and specific staining appears as brown-stained cytoplasm.

To determine specificity of staining, three controls were used: (i) adsorbed immune serum (1 h, 37°C) with 10-fold molar excess of the specific peptides for the ICE antibodies (#sc-1780 P; Santa Cruz), the rhIL-1β (Genzyme, Cambridge, MA) and rhIL-18 (Santa Cruz); (ii) omission of the primary antibody; and (iii) substitution of the primary antibody with IgG (Nordic Immunology, Tilburg, The Netherlands) following the same experimental protocol.

STATISTICAL ANALYSIS

The data are expressed as a mean±SEM. Statistical significance was assessed by the paired Student's *t*-test, and $P<0.05$ was considered significant.

Results

EFFECT OF DIACERHEIN AND RHEIN ON ICE EXPRESSION

The effect of the drugs on ICE mRNA levels was first examined on OA chondrocytes using Northern blotting analysis ($N=3$). Generally, on these cells only one transcript of 1.9 kb is detected. When a very high level of ICE mRNA is present, a second transcript of 2.5 kb could be seen. As illustrated in Fig. 1, diacerhein and rhein at 20 µg/ml has no true effect on ICE mRNA levels.

Since the Northern blotting reflects the level of expression in chondrocytes from the whole cartilage population, further experiments using in-situ hybridization were then performed permitting the cellular localization of ICE expression. Results ($N=7$) revealed, as expected,[22] that in OA, ICE is expressed throughout the entire cartilage thickness, with a greater number of chondrocytes staining positive in the superficial zone compared with the deep zone; percentages of 36.4±5.0% and 21.3±5.8% ($P<0.001$) were found respectively (Fig. 2). Both diacerhein and rhein only slightly decreased cell scores, with a higher effect at the superficial zone (Fig. 2). However, this did not reach statistical significance.

EFFECT OF DIACERHEIN AND RHEIN ON ICE SYNTHESIS

Data from the specific ICE ELISA assay ($N=6$) revealed that OA chondrocytes produced a large amount of this enzyme (246.9±27.2 pg/5×10^5 cells). Diacerhein and rhein, at both concentrations used (20 and 10 µg/ml) induced a statistically significant decrease in ICE protein levels (Fig. 3). A mean reduction of about 34% was found for diacerhein and 45% for rhein.

According to a previous immunohistochemical study,[22] a statistically higher percentage of chondrocytes stained positive for ICE protein at the superficial zone than at the deep zone. As illustrated in Fig. 4, results from this study agreed with the previous ones in which a statistically significant greater ($P<0.02$) number of positive cells were found in the superficial zone (Fig. 4). Diacerhein and rhein ($N=7$) induced a marked and statistically significant decrease in the number of cells staining positive for ICE.

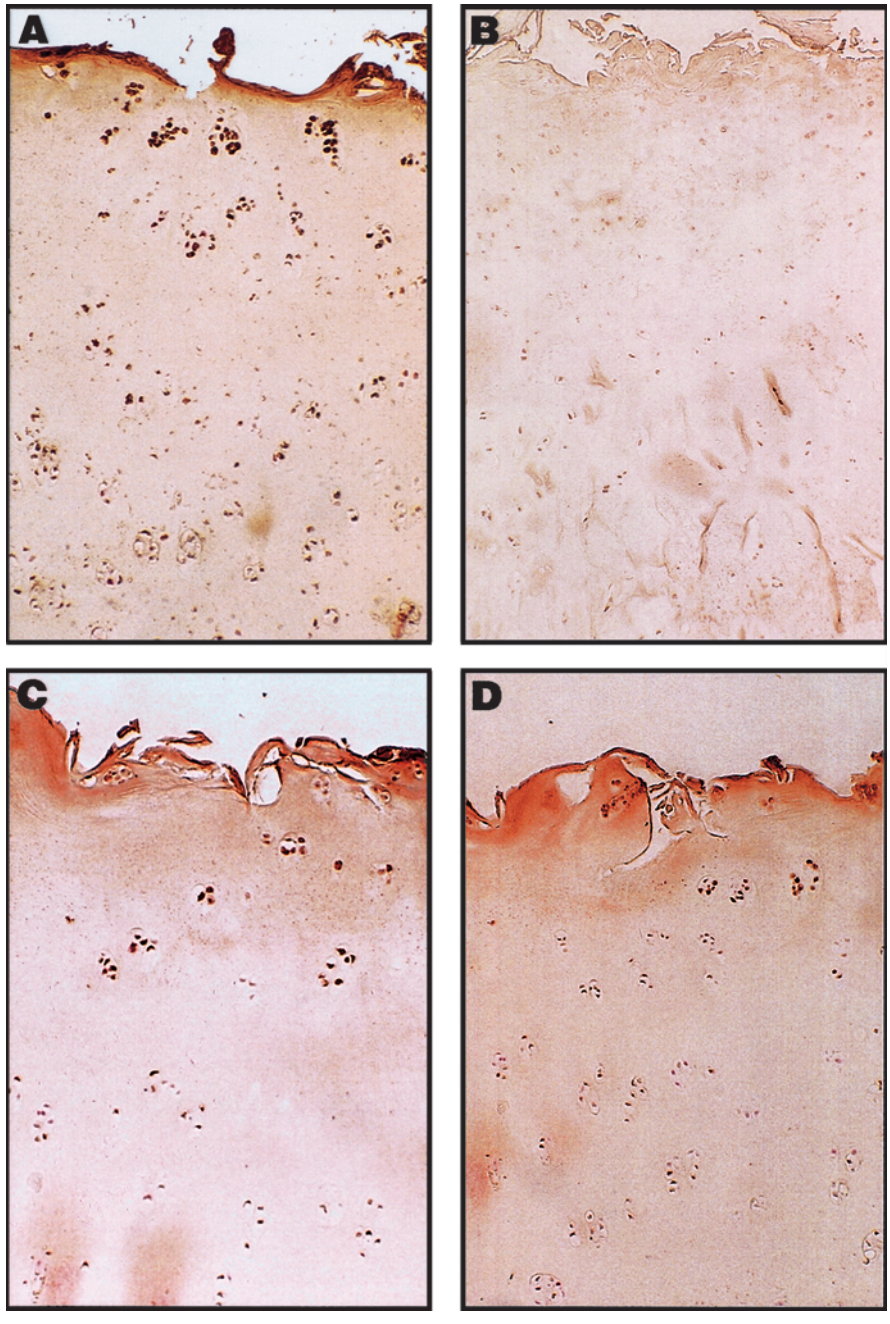

Fig. 4A–D.

This effect was found for both the superficial and deep zones of the cartilage.

EFFECT OF DIACERHEIN AND RHEIN ON ACTIVE IL-1β AND IL-18 PRODUCTION

Additional experiments were carried out to evaluate the effects of both agents on ICE toward its specific substrates, IL-1β and IL-18. Both IL-1β and IL-18 were detected in all OA cartilage specimens examined (N=7).

In the untreated cartilage, staining for IL-1β was found predominantly in chondrocytes at the superficial zone. In this zone, 9.5±1.4% of the cells were immunoreactive compared to 2.1±0.8% (P<0.0001) in the deep zone (Fig. 5). The diacerhein- and rhein-treated cartilage showed a marked decrease in the number of chondrocytes staining positive for IL-1β regardless of the cartilage zones. In the superficial zone, a significant reduction in the cell score was found when treated cartilage was compared to control (untreated) (diacerhein, P<0.009; rhein, P<0.003). A significant reduction (P<0.01) was also found for rhein-treated cartilage at the deep zone.

Immunostaining for IL-18 demonstrated a cellular distribution pattern similar to that described for IL-1β. However, a larger number of chondrocytes stained positive for IL-18 when compared with IL-1β. Cell scores of 40.9±4.6% and 21.4±3.1% (P<0.0005) were found at the superficial and

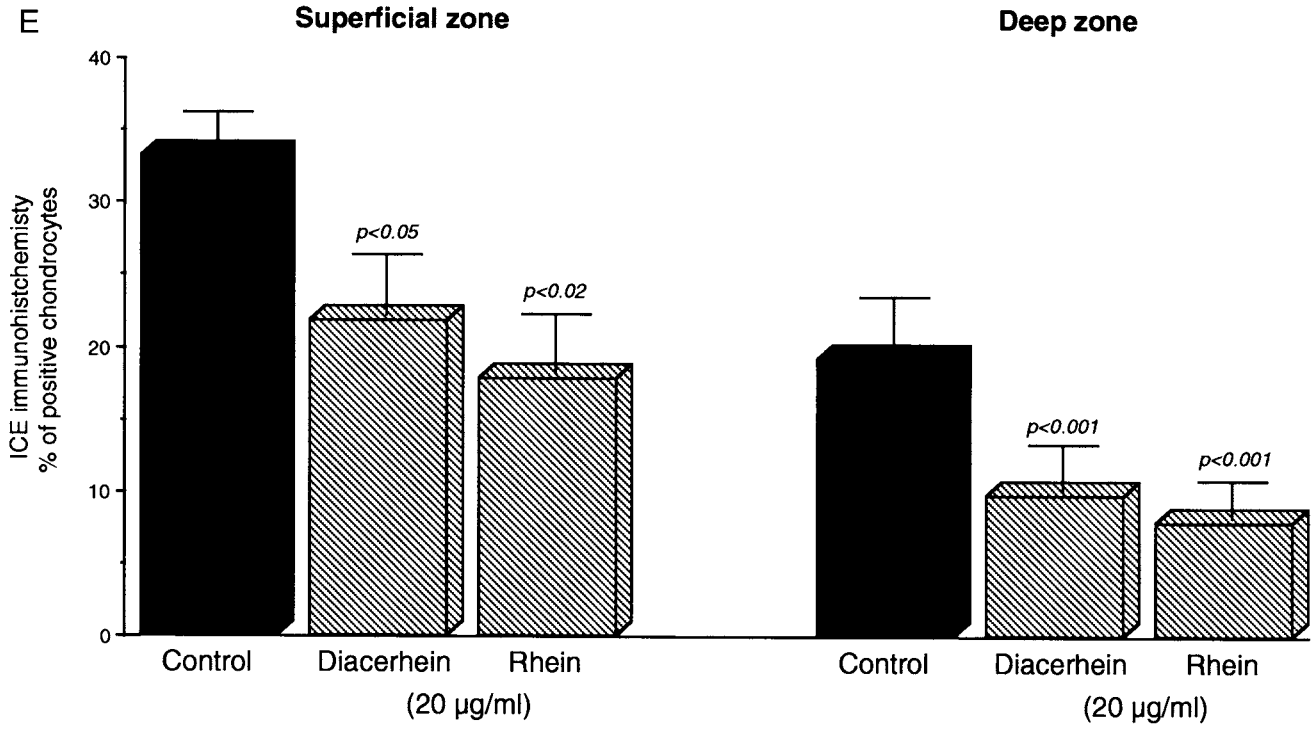

Fig. 4. Representative sections of ICE immunostaining in human OA cartilage specimens (A). (B) represents the immunohistology done on cartilage incubated with the adsorbed immunoserum as control. In (C) and (D), OA cartilage was treated with diacerhein (C) or rhein (D) at 20 µg/ml. (Original magnification ×63.) (E) Histogram of ICE immunostaining score in human OA cartilage from the superficial (superficial and upper intermediate layers) and deep (lower intermediate and deeper layers) zones. Data are expressed as the mean±SEM cell score. Statistical analysis revealed a significant difference (P<0.02) between the OA controls from the superficial and deep zones, and P-values indicate comparison with the OA control.

deep zones respectively (Fig. 5). Both diacerhein and rhein induced a significant (P<0.02, P<0.04 respectively) reduction of IL-18 production at the superficial zones, and for rhein (P<0.009) in the deep zones.

Discussion

Morphological changes observed in OA include cartilage erosion and varying degrees of synovial inflammation.[8] It is known that proinflammatory cytokines, locally produced by pathological articular joint tissues, contribute to these alterations. Studies on the contribution of cytokines to the cartilage destruction in OA, however, point to a prime role played by IL-1β.[1–7] Perpetuation of the degradative process in articular joint tissue appears, at least in part, dependent on the level of the active cytokine. Therefore IL-1β activity represents an important therapeutic target.

IL-1β is synthesized as a precursor that undergoes proteolytic cleavage by ICE, permitting the activated cytokine to exit the cell. The potential role of ICE in several pathophysiological systems has been the focus of much attention in the last few years. Several in-vitro, and particularly in-vivo studies using ICE deficient mice have demonstrated that this enzyme plays a key role in the maturation (activation) process of IL-1β but also in another cytokine, IL-18.[14–17] Hence, *in vivo*, the action of ICE on these cytokines appears to be a key limiting factor for these cytokines to be secreted by the cell.

Results from several studies have shown that diacerhein and rhein are potent inhibitors of proinflammatory cytokine synthesis and activity, particularly for IL-1β.[29,31] Moreover, *in vitro* diacerhein and rhein demonstrated their ability to inhibit IL-1β-induced collagenase synthesis by chondrocytes, the IL-1β-induced NO production and to partially reverse the IL-1β-induced inhibition of proteoglycan synthesis.[26–28,30] We hypothesize that the effect on IL-1β could have occurred through, at least in part, the maturation process involving the inhibition of ICE. Our data showed that diacerhein and rhein markedly inhibit ICE production as well as the activation processing of IL-1β and IL-18 *in vitro* in chondrocytes and *ex vivo* in cartilage explants.

Our laboratory previously observed the presence of a significant up-regulation of ICE, IL-1β and IL-18 in human OA cartilage.[22] The present finding of an inhibition of ICE by drugs is of particular importance, since ICE is known to have a pivotal role in the maturation process of IL-1β in OA cartilage, one of the cytokines believed to play a predominant role in the progression of the disease's structural changes. The up-regulation of ICE levels in OA tissues was demonstrated to be present both at the synovial membrane and cartilage.[22] In the synovium, the concomitant increase in the expression of ICE and active IL-1β favors an important role of ICE as one of the main mechanisms by which excess production of mature IL-1β can occur. IL-1β could then, in an autocrine/paracrine fashion, exert its local effects on OA cells by inducing an excess in production of proteases, nitric oxide and other catabolic factors responsible for joint structural damages. At the cartilage level, the simultaneous increase in production of both IL-1β and ICE

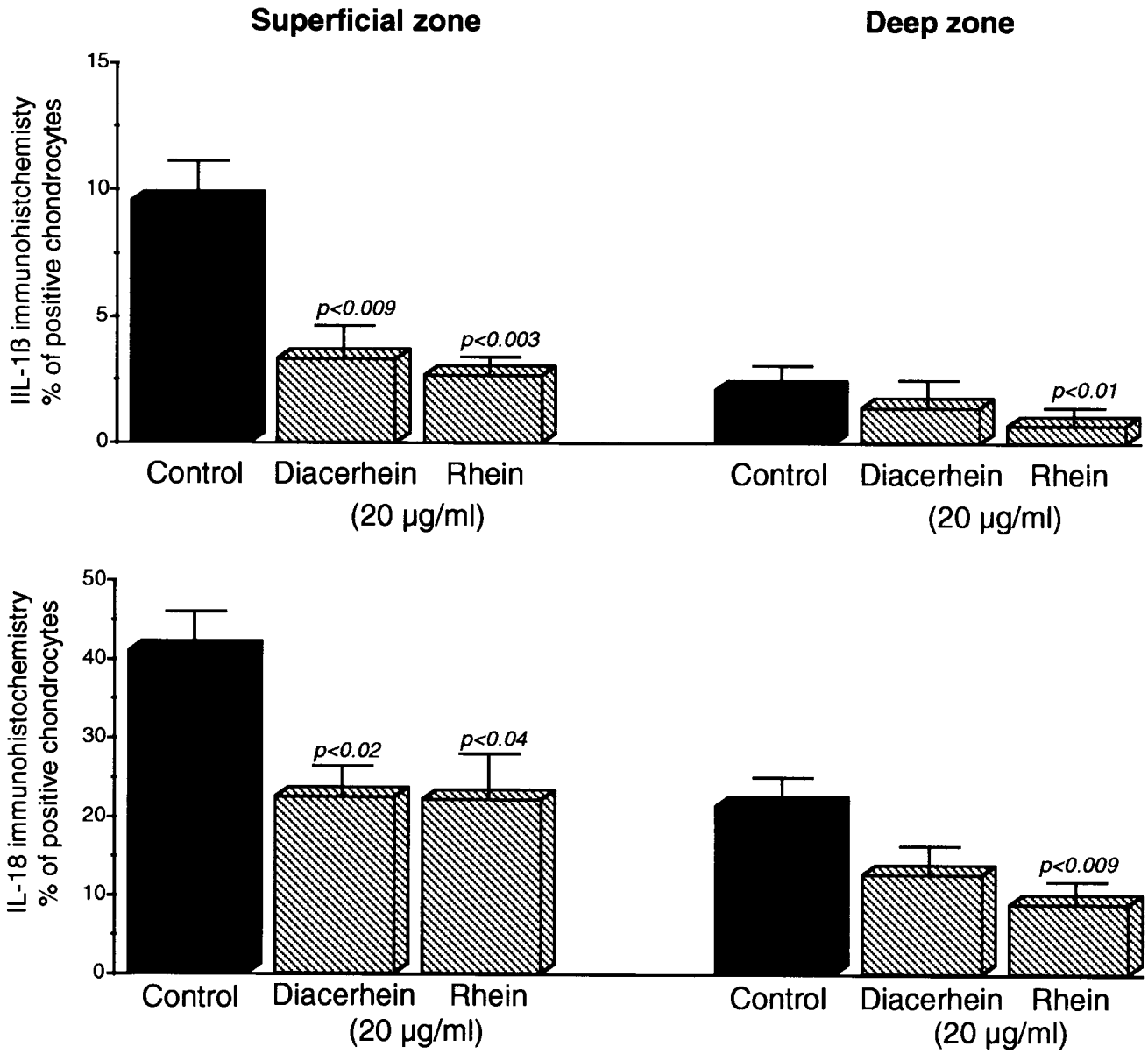

Fig. 5. Histogram of the immunostaining score for IL-1β (top panel) and IL-18 (bottom panel) in OA cartilage from the superficial (superficial and upper intermediate layers) and deep (lower intermediate and deeper layers) zones when human OA cartilage were incubated in the absence (control) or presence of diacerhein or rhein at 20 µg/ml. Data are expressed as the mean±SEM cell score. Statistical analysis revealed a significant difference between the OA controls from the superficial and deep zones for IL-1β ($P<0.001$) and IL-18 ($P<0.005$), and P-values indicate comparison with the OA control.

in OA chondrocytes[22] makes it tempting to hypothesize that ICE is a key factor responsible for the local increase in the level of mature IL-1β in this tissue.

The action of diacerhein and its active metabolite rhein did not appear to occur through the reduction of total ICE mRNA. Another possibility, although speculative, is that these drugs could act on ICE mRNA half-life. Yet, this was not demonstrated in any of the factors affected by diacerhein/rhein. In this study, however, we showed that these drugs reduce ICE production. By immunohistochemistry, the reduction of ICE protein levels in OA cartilage was found both at the superficial and deep zones, demonstrating a good diffusion of the drugs in the matrix and also suggesting a similar sensitivity of the chondrocytes from both zones to the drug. This study and previous reports clearly demonstrate that diacerhein and rhein are very potent agents to reduce *in vitro* the action played by the IL-1 system in OA cartilage. The in-vivo effect of diacerhein on the IL-1 system is therefore several-fold, since this drug inhibited the global synthesis of IL-1β, its maturation, and the IL-1 receptor levels on OA cells.[29] Consequently, diacerhein could produce *in vivo* a complete inhibition of the IL-1β effect. It is therefore not surprising that, *in vivo*, diacerhein was shown to reduce the severity of morphological changes in the experimental dog model of OA, since IL-1β has been shown to play a predominant role in the progression of the structural changes.[4,45,46] In the matter of diacerhein being a potential disease-modifying effector, it

was recently shown that this drug could also favor cartilage repair, as it induces TGF-β1 and TGF-β2 expression in chondrocytes.[47] In another context, some data in the literature suggests that TGF could upregulate ICE production.[48] However, and depending on the cell system used, contrasting data has been reported.[49,50]

IL-18 was identified as a consequence of its ability to induce interferon-γ (INF-γ) production in mice following endotoxin shock.[51,52] IL-18 shares features of the IL-1 signature-like sequence. This cytokine shares a 12β sheet structure in common with that of the IL-1 family. However, IL-18 does not share substantial identity with the regions of IL-1β which interact with the IL-1 receptor.[53] Only limited tissue distribution for IL-18 has been performed, however, IL-18 mRNA appears to be widely but not universally expressed. Moreover, we and others have recently shown that this cytokine is expressed and produced by human chondrocytes and its level increased in OA cells.[22,54] Although its role in pathologic cartilage still remains to be determined, it was recently demonstrated that IL-18 induces proinflammatory and catabolic responses in cartilage.[54] The results of the present immunohistochemical study clearly show that both diacerhein and rhein could effectively reduce the level of IL-18 in OA cartilage chondrocytes, and that this effect is noted in all layers of cartilage. The nature of the antibody that was used in this study did not allow us to discriminate whether the decrease was due to a reduction in the synthesis and/or maturation of IL-18, since the antibody recognized both forms of the cytokine. However, the marked reduction in the level of ICE induced by diacerhein/rhein is strongly suggestive of a reduction in, at least, the level of the active form of IL-18.

Together, these data indicate that diacerhein and rhein can suppress the ICE production and therefore, the presence of active forms of two pro-inflammatory cytokines, IL-1β and IL-18, and provide additional information about the mechanism of action of diacerhein and rhein by which it may reduce the progression of the structural changes of OA.

Acknowledgments

The authors thank François Mineau and Kayghobad Kiansa for their invaluable technical expertise, and Shirley McCarthy for her assistance in manuscript preparation.

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